A Leishmaniasis Vaccine Breakthrough: Are We Almost There?

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Simple Cutaneous Leishmaniasis

- Caused by *Leishmania major, mexicana, tropica, etc*
- Normally self-healing
- DTH, T cell proliferation
- Low antibody responses
- Healing results in solid immunity
Diffuse cutaneous leishmaniasis

- No DTH response
- High Antibody response
- Chronic, resistant to drug treatment
Mucocutaneous leishmaniasis

- Very strong DTH
- Intense inflammatory response
- Chronic, resistant to drug treatment
- Very few parasites at inflammatory sites
Visceral leishmaniasis

- *Leishmania donovani*
- No DTH response
- Very high Ab response
- Impaired T cell proliferation in response to antigen
Leishmaniasis: A global problem

Epidemiology:
- 14 million active cases
- 2 million new cases yearly
- 340 million people at risk
- 88 countries, 5 continents
- Neglected Tropical Disease
- > 3500 cases of cutaneous Leishmaniasis among gulf veterans

Sources: WHO, CDC
Immunity to cutaneous Leishmaniasis (simplified)

- Resistance to *L. major* is mediated by IFN-γ-producing CD4+ Th1 cells
- IFN-γ activates macrophages leading to intracellular parasite killing via:
  - Nitric oxide (NO) production
  - ROI and RNI radicals

Summary:
- CD4+ Th1 cells
- IFN-γ production
Infection-induced immunity

- Healing of cutaneous leishmaniasis in humans and mice is usually self-resolving
- This is associated with long-lasting resistance to reinfection

✓ Suggests existence of anti-Leishmania memory cells
**CD4^+ T cells mediate infection-induced immunity**

**Infection-induced Resistance**

- **Primary**
- **Secondary**

![Graph showing infection-induced resistance](graph)

**Adoptive Transfer of Protection**

- **No Cells**
- **Naïve Cells**
- **Immune CD4^+ T Cells**

![Graph showing adoptive transfer of protection](graph)
Is there a need for a *Leishmania* vaccines?

- **Epidemiology:**
  - ✓ Over 14 million active cases
  - ✓ 2 million new cases yearly
  - ✓ 340 million people at risk
  - ✓ 88 countries, 5 continents

- Chemotherapy until recently is very limited high cost, toxicity and route of administration

- Elimination can likely only be achieved through widespread vaccination

- Vaccination could provide longterm reductions in potential reservoirs
Pubmed Search!!

• Leishmania and immunity = 2606
• Leishmania and resistance = 2127
• Leishmania and vaccine = 1649
• Leishmania and vaccination = 689
• Leishmania and effective vaccine = 392
• Human Leishmaniasis and effective vaccine = 227
• Leishmania vaccination trials = 115

Approved Effective Human *Leishmania* Vaccine = 0
Scientific Issue: Why is there no effective human vaccine despite prolific publications?
• We do not fully understand the correlates of protective immunity.

✓ Immunologic memory (development, maintenance and loss)

✓ Antigens that mediate memory T cell responses
Is Vaccination Feasible?

• Infection-induced immunity: Recovery from virulent *Leishmania* infection leads to development of strong durable immunity against virulent challenge

• Leishmanization is very effective
Leishmaniasis Vaccine Approaches

- Heat Killed parasites
  - First generation vaccines

- Live attenuated/genetically modified
  - Second generation vaccines

- Defined protein/subunit vaccines
  - Third generation vaccines

- Leishmanization
Heat-killed (ALM) vaccines

- Heat-inactivated (Autoclaved) *Leishmania* parasites
- Several doses or ‘boosters’ with different adjuvants (BCG, Alum, CpG)
- Questionable efficacy
- Easy and cheap to make (minimal technology) and very safe
- Does not require understanding of complex immunologic correlates of protective immunity
- Reports of immunotherapeutic effects in New World cutaneous leishmaniasis
Problems with ALM Vaccine

• Standardization and scale-up issues

• Undesirable immune responses

Leishmanization

• Deliberate inoculation of live (virulent) parasites to the hidden parts of the body with the aim of inducing protection from natural infection following recovery

• Very Effective

• Undesirable side effects!!!
Undesirable effects of Leishmanization

Courtesy: Dr Ali Khamispour
Rationale for Live Vaccine

• Infection-induced Immunity
  ✓ Recovery from infection leads to development of durable immunity

• Persistent parasites are important for maintaining infection-induced immunity
  ✓ Attenuated parasites persist for a long time in infected/vaccinated host
  ✓ Clearance of parasites leads to loss of protective immunity

• Leishmanization works
Two major strategies for live-attenuated vaccine:

• Virulent parasites (*Leishmanization*)

• Attenuated parasites
Attenuated Parasites

- **Gamma Irradiation**
  - Rivier et al., 1993

- **Serial *in vitro* passage with/without drugs**

- **Infection with non-pathogenic strain (*L. tarentolae*)**
  - Breton et al 2005

- **Genetically modified organisms**
  - LPG1, LPG2, LPG5A/B, DHFR-Ts, GDP-MP, GP63, etc
LPG2 KO parasites do not cause pathology despite persistence

Add back
lpg2-
WT

Lesion size (mm)

Weeks post-infection

Uzonna et al 2004
Vaccination with LPG2 KO parasites induce protection against virulent challenge

Uzonna et al 2004
Problems of live-attenuated vaccine

• **Reversion to virulence** (due to long-term persistence)

• **Contraindicated in immuno-compromised individuals**

This vaccination protocol will most likely never be approved
3rd Generation Vaccines

• Subunit vaccines with refined products (recombinant proteins)

• Several recombinant proteins or polyproteins (e.g. Leish-111F, LmSTI1, KSAC, etc) with adjuvants such as MPL

• Some have progressed into clinical trials

• One approved for dogs in South America (Leishmune)
Why 3\textsuperscript{rd} Generation Vaccines Fail

• Use of \textit{in silico} models to predict \textquotedblleft protective\textquotedblright\ vaccine candidates

• Selection of candidates based primarily on those that elicit \textbf{strong primary immune response}

• Skewed and biased immunization protocols: designs favor publication; not necessarily long-term protection
  – Animal model (BALB/c mice)
  – Antigen dose
  – Type of adjuvants
  – Frequency of immunization
  – Time of challenge etc.
Objectives:

• To use \textit{in vivo} approach to identify \textit{Leishmania} antigens that induce infection-induced immunity (memory CD4$^+$ T cell responses?)

• Assess whether vaccination with these antigens will induce long-lasting protection against virulent challenge
Healing from primary *L. major* infection is associated with strong antigen-specific CD4⁺ T cell proliferation and IFN-γ production.
Premise: Identification of the antigens that induce and maintain infection-induced resistance and the corresponding T cells is critically important for development of effective vaccine and vaccination strategy against leishmaniasis.
Strategy to confirm stimulatory ability of *L. major*-infected BMDCs for CD4⁺ T cells

1. Purify CD4⁺ T cells
2. Co-culture with infected or uninfected BMDCs
3. Assess:
   1. Proliferation
   2. IFN-γ production

Flow Cytometry
L. major-infected DCs induce proliferation and IFN-γ production in CD4+ T cells from healed mice

Source of T cells

<table>
<thead>
<tr>
<th>Source of APC</th>
<th>Naïve Mice</th>
<th>Healed Mice</th>
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<tbody>
<tr>
<td>Uninfected DC</td>
<td>0.8 ± 0.1</td>
<td>0.7 ± 0.3</td>
</tr>
<tr>
<td>Infected DC</td>
<td>3.0 ± 1.8</td>
<td>6.3 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>0.7 ± 0.3</td>
<td>6.5 ± 1.9**</td>
</tr>
<tr>
<td></td>
<td>1.9 ± 0.4</td>
<td>24.7 ± 3.9</td>
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</table>
Proteomic identification of *L. major* antigenic peptides that bind to MHC II molecules

**Infected BMDCs** → **Lysis buffer** → **Anti-MHC II Ab** → **Protein A beads** → **Dissociate MHC/Peptide** → **Membrane cutoff: 10K** → **Lyophilization**

**Programs:**
- GPM
- ProteinPilot

**Databases:**
1. *L. major*
2. Mouse

**Database Searching** → **QSTAR® Elite Hybrid LC/MS/MS** → **MHC-II bound Peptides**
<table>
<thead>
<tr>
<th>Source Protein</th>
<th>accession</th>
<th>Mr</th>
<th>Log(e) in GPM</th>
<th>Sequence in GPM</th>
<th>Sequence in ProteinPilot</th>
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<tr>
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<td>Putative uncharacterized protein</td>
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<td>Glycosomal phosphoenolpyruvate carboxykinase, putative</td>
<td>LmjF27.1805</td>
<td>58.2</td>
<td>-21.4</td>
<td>NDAFGVMPPVARLTPEQ; DAFGVMPPVARLTPE; DAFGVMPPVARLTPEQ; DAFGVMPPVARLTPE</td>
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<td>Dihydrolipoyl dehydrogenase</td>
<td>LmjF32.3310</td>
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<td>Cytochrome c</td>
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<td>Aconitase, putative</td>
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Synthetic (PEPCK\textsubscript{335-351}, P3) peptide stimulates proliferation and IFN-\(\gamma\) production by CD4\(^+\) T cells from healed mice

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<th>Sequence</th>
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<tr>
<td>P1</td>
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<td>P2</td>
<td>AAEASAHSPQASQSGDG</td>
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<td>P3</td>
<td>NDAFGVMPVPVARLTEEQ</td>
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<tr>
<td>P4</td>
<td>APAPAPAAAAPTPASAPVS</td>
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- **PEPCK**: phosphoenolpyruvate carboxykinase
- Plays an essential role in glyconeogenesis

Sci Transl Med. 2015 Oct 21;7(310)
Expansion, contraction and maintenance of PEPCK-specific CD4+ T cells in *L. major*-infected mice

Sci Transl Med. 2015 Oct 21;7(310)
PEPCK-specific CD4+ T cells are polyfunctional effector cytokine producers

Sci Transl Med. 2015 Oct 21;7(310)
PEPCK-specific CD4$^+$ T cells Protect naïve mice against *L. major* challenge

![Diagram](image)

- **a**
  - Pre-sort
  - Post-sort

- **b**
  - Parasite burden (log$_{10}$)
    - CD4$^+$Tet$^+$ (2×10$^4$)
    - CD4$^+$Tet$^-$ (2×10$^5$)
    - CD4$^+$Tet$^-$ (4×10$^6$)
    - Naive CD4$^+$ (4×10$^6$)

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Sci Transl Med. 2015 Oct 21;7(310)

38
Phosphoenolpyruvate carboxykinase (PEPCK) is expressed by promastigotes and amastigotes.

**a**

<table>
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<tr>
<th>100 kD</th>
<th>65 kD</th>
<th>50 kD</th>
<th>40 kD</th>
<th>30 kD</th>
<th>25 kD</th>
<th>20 kD</th>
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</table>

**b**

- Promastigote
- Amastigote
- 10ng rPEPCK
- 50ng rPEPCK

**c**

- DAPI
- PEPCK
- DAPI/PEPCK
- White light

Promastigote

Amastigote
PEPCK elicits strong T cell response in *L. major*-infected healed human patients

**a**

**Index of stimulation**

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**b**

**IFN-γ (ng/ml)**

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**c**

**Grz B (ng/ml)**

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</table>
Does Vaccination with PEPCK Protect Against Virulent Challenge?

**DNA Vaccine:**
1) PBS
2) CpG control
3) DNA + CpG
4) Vector + CpG

**Protein Vaccine:**
1) PBS
2) CpG control
3) Protein + CpG
4) Protein only

**Peptide Vaccine:**
1) PBS
2) CpG control
3) Peptide + CpG

**Challenge:**
- L. major
- L. donovani

**Immune response**
Vaccination with the synthetic peptides and DNA vaccine expressing PEPCK induces protection against virulent *L. major* challenge.
Vaccination with the recombinant PEPCK induces protection against virulent *L. major* challenge. (6 weeks)
DNA Vaccine Confers Long-term (12 Weeks) Protection

**A** C57BL/6

- PBS
- CpG
- Vector
- DNA vaccine

Lesion size (mm) vs. Weeks Post-challenge

**B** Balb/c

- PBS
- CpG
- Vector
- DNA vaccine

Lesion size (mm) vs. Weeks Post-challenge

**C**

Parasite burden (log_{10}) vs. PBS, CpG, Vector, DNA vaccine

**D**

Parasite burden (log_{10}) vs. PBS, CpG, Vector, DNA vaccine
PEPCK Vaccine Cross-Protects Against Experimental Visceral Leishmaniasis

**LIVER**

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<td>DNA vaccine</td>
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**SPLEEN**

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<td>Vector</td>
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<td>DNA vaccine</td>
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**Summary:**

Naturally processed *Leishmania major* peptides were eluted and identified from MHC II molecules on *L. major*-infected BMDCs.

PEPCK-specific T cells are immunodominant, polyfunctional cytokine producers and protect against *L. major*.

Vaccination with PEPCK peptides, PEPCK DNA or rPEPCK induces cross-protection against *Leishmania* challenges.

- *L. major, L. donovani, L. infantum* (preliminary evidence)
Implications for Vaccine Designs and Vaccination Strategies

• Conventional methods of immunogen prediction may be misleading
  ✓ *In silico* predictions, early versus memory immune responses

• Approach aimed at identifying antigens that are really relevant
  ✓ *Reverse immunology*

• May be relevant in other parasitic infections where concomitant immunity is critical for resistance
  ✓ *Malaria, toxoplasmosis*
Next Step:

• Vaccination studies in NHP, Dogs
• Clinical Trials?
• Collaborators and funding wanted!!!
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